

(TALM) [1]. FOF1 ATP synthase had a distinct trajectory map from Tom20, and was frequently restricted in cristae. We screened the localization and mobility maps of various other mitochondrial proteins. Their distribution in the outer mitochondrial membrane, the intermembrane space, the inner mitochondrial membrane and the matrix was concordant with previous biochemical/topographic data. The present work revealed their respective mobility profiles. In principle, our data support the random collision model of OXPHOS complexes. In the context of metabolic adaption, aging and disease trajectory maps might serve as a diagnostic for changes in localization and mobility related to (ultra-)structural variations. In sum, TALM is a versatile tool to dissect the localization, distribution and mobility of (mitochondrial) proteins in live cells and visualize the accessible micro-compartments without destruction of biological material.

## Reference

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## S2.02

### Mitochondrial nucleoids as revealed by 3D super-resolution microscopy

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The current view of complexes of double-stranded circular molecules of mitochondrial (mt) DNA with accessory proteins and mtDNA genetic machinery proteins is still very preliminary and scarce. These complexes, called nucleoids, are traditionally studied by 2D confocal microscopy having a resolution higher than 250 nm, even though the expected average size of mt nucleoids is about 100 nm [1]. Mitochondrial nucleoids were frequently visualized using immunocytochemistry as <1000 foci per cell in cultured cells. 3D superresolution fluorescent photoactivatable localization microscopy (FPALM) [1], in conjunction with photoconvertible fluorescent protein conjugates of marker proteins such as mitochondrial (mt) transcription factor A (TFAM) and mt single-stranded-DNA-binding protein (mtSSB), has provided evidence that nucleoids in hepatocellular HepG2 cells exhibit a rather wide size distribution ranging between 50 and 300 nm. This was confirmed using 3D TFAM/mtSSB immuno-cytochemistry in conjunction with the direct stochastic optical reconstruction microscopy (dSTORM). Moreover, using 3D double color FPALM/dSTORM microscopy, we have positioned mtSSB foci within the nucleoid space represented by TFAM or mtDNA and demonstrated that mtSSB is concentrated in these smaller spaces. We have also positioned mtDNA nucleoids within the mitochondrial reticulum network visualized by dSTORM 3D immunocytochemistry of TIM23. In parallel, 3D 4Pi microscopy of mtGFP with Alexa 647/mtSSB immunostained nucleoids was performed. These results demonstrated nearly equidistant ~1 µm inter-nucleoid distances and confirmed our hypothesis on nucleoid redistribution within the

fragmented network [2] – clustering of up to nine nucleoids in 2 µm diameter mitochondrial spheroids of a fragmented mt network – arising from an original 10 µm mitochondrial tubule. Supported by GACR grants no. 13-02033, P305/12/1247.

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## S2.03

### Cardiolipin is a key determinant for mitochondrial DNA stability and segregation during mitochondrial stress

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Mitochondria play a key role in adaptation during stressing situations. Cardiolipin, the main anionic phospholipid in mitochondrial membranes, is expected to be a determinant in this adaptive mechanism since it modulates the activity of most membrane proteins. Here, we used *Saccharomyces cerevisiae* subjected to conditions which affect mitochondrial metabolism as a model to determine the possible role of cardiolipin in stress adaptation. Interestingly, we found that thermal stress promotes an increase in cardiolipin content, modifying both surface charge and the physical state of mitochondrial membranes. These changes have effects on mtDNA segregation and mitochondrial morphology, thus, adapting cells to thermal stress. Conversely, this effect is cardiolipin-dependent since a cardiolipin synthase-null mutant strain is unable to adapt to thermal stress. Interestingly, we found that the loss of cardiolipin specifically affects the segregation of mtDNA to daughter cells, thus leading to a respiratory deficient phenotype after replication. Overall, our results demonstrate that the mitochondrial lipid cardiolipin is a key determinant in the maintenance of mtDNA stability, morphology and segregation.

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## S2.P1

### Molecular mechanisms for the induction of peroxidase activity of the cytochrome c–cardiolipin complex

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Cardiolipin (CL) is a unique phospholipid in mitochondrial membranes with diverse biological functions. It is becoming evident that CL